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APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR / PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
09235875	1/22/1999	MADISON ET AL.	MBX020

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ART UNIT	**PAPER**
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Commissioner for Patents

Section (8) of the Examiner's Answer has been amended to include references from section (9) not listed in the previous version of the Examiner's Answer.

U.S. Patent 5,470,727 (Macharenas et al.);
U.S. Patent 5,512,482 (Voelker et al.);
Boynton Z. et al. (J. of Bacteriology June 1996, Vol. 178, No. 11, p. 3015-3024);

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Russell Kallis whose telephone number is (571) 272-0798. The examiner can normally be reached on M-F 8:30-5.
If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anne Marie Grunberg can be reached on (571) 272-0975. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.
Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Russell Kallis Ph.D.
June 8, 2007

RUSSELL P. KALLIS, PH.D.
PRIMARY EXAMINER

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BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Application Number: 09/235,875
Filing Date: January 22, 1999
Appellant(s): MADISON ET AL.

Patrea L. Pabst
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed 12/11/2006 appealing from the Office action mailed 2/10/2006.

(1) Real Party in Interest

A statement identifying by name the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The statement of the status of claims contained in the brief is correct.

(4) Status of Amendments After Final

The Appellants' statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Claimed Subject Matter

The summary of claimed subject matter contained in the brief is correct.

(6) Grounds of Rejection to be Reviewed on Appeal

The Appellants' statement of the grounds of rejection to be reviewed on appeal is substantially correct. The changes are as follows:

Claims 37-39 were previously rejected under 112 1st paragraph as canceled original claims 24-26 are now rejected under 112 1st written description.

Claim 39 is rejected under 112 2nd paragraph for lacking antecedence.

The rejection under 102(b) as being anticipated by Fukui *et al.* (J. of Bacteriol. Vol. 179: 4821-4830, 1997) has been modified for clarity and encompasses supporting art offered as better evidence that limitations recited in the claim are inherent to the material or were known features of the composition required to practice the method and to address newly filed claims 35-39. Claims 10 and 14 are now included in the rejection under 102(b) as well as new claims 36 and 38.

The rejection under 103(a) as being unpatentable over Timm A. *et al.* (Applied and Environmental Microbiology, November 1990, Vol. 56, No. 11, p. 3360-3367) in view of Fukui *et al.* (J. of Bacteriol. 1997; Vol. 179: p. 4821-4830) and in further view of Hoffman N. *et al.* (FEMS Microbiology Letters, 2000, p. 253-259); and the rejection under 35 U.S.C. 103(a) as being unpatentable over Schubert P. *et al.*, (J. of Bacteriology 1988; Vol. 170, No. 12, p. 5837-5847) in view of Fukui *et al.* (J. of Bacteriol. Vol. 179: 4821-4830, 1997) in further view of Boynton Z. *et al.* (J. of Bacteriology June 1996, Vol. 178, No. 11, p. 3015-3024) and Feigenbaum J, *et al.* (PNAS, February 1977; Vol. 74, No. 2, pp. 492-495) are withdrawn in response to Appellants' arguments and to simplify the issues.

The former rejection under 35 U.S.C. 103(a) as being unpatentable over Fukui *et al.* (J. of Bacteriol. 1997; Vol. 179: p. 4821-4830), in view of Macharenas *et al.* (U.S. Patent 5,470,727 issued 28 November 1995); in further view of Schubert P. *et al.*, (J. of Bacteriology 1988; Vol. 170, No. 12, p. 5837-5847) has been restructured to further incorporate Boynton Z. *et al.* (J. of Bacteriology June 1996, Vol. 178, No. 11, p. 3015-3024) as well as Appellants' specification.

The copy of the appealed claims contained in the Appendix to the brief is correct.

(7) Claims Appendix

The copy of the appealed claims contained in the Appendix to the brief is correct.

(8) Evidence Relied Upon

Schubert P. *et al.*, (J. of Bacteriology 1988; Vol. 170, No. 12, p. 5837-5847);

Haywood G.W. *et al.* FEMS Microbiology Letters; 1988, Vol. 52 pp. 91-96;

U.S. Patent 6,586,658 (Peoples *et al.*);

U.S. Patent 5,470,727 (Macharenas *et al.*);

U.S. Patent 5,512,482 (Voelker *et al.*);

Boynton Z. *et al.* (J. of Bacteriology June 1996, Vol. 178, No. 11, p. 3015-3024);

Haywood G.W. *et al.* FEMS Microbiology Letters; 1988, Vol. 52 pp. 259-264;

Doi Y. *et al.* Applied Microbiology and Biotechnology; 1988, Vol. 28 pp. 330-334;

Kremer, L. *et al.* J. of Biol. Chem. July 27, 2001; Vol. 276, No. 30, pp.27967-27974;

GenBank Accession gi: 113527306 for a FabD gene encoding the *Ralstonia eutropha*;

GenBank Accession gi: 73539398 for an acyl CoA synthase (i.e. synthetase) gene from *Ralstonia eutropha*;

Malonyl-CoA structure from Concise Encyclopedia Biochemistry 2nd. Ed. Scott, T. and Eagleson, M.; Walter de Gruyter: Berlin-New York 1988 on page 197;

and from Appellants's specification:

page 11 lines 13-16 and 22-24 for Haywood references above;

example 4 pages 24-25 recites Fukui *et al.*, J. of Bacteriol. Vol. 179: 4821-4830, 1997 for the source of the D-specific hydratase of claim 16; and

page 14 line 26 to page 15 line 1 that points to *R. eutropha* (*A. eutrophus*) as a source of an (FaoAB) complex of claim 36 that has the epimerase activity of claim 38.

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claims 1, 6-7, 10, 14, 16-21 and 35-39 are pending and examined.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 37-38 and 39 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

The claims are drawn to methods requiring expression in bacteria of unspecified fatty acid biosynthetic enzymes from *Norcardia salmonicolor*, an enzyme that epimerizes S-3-hydroxyhexanoyl-CoA to R-3-hydroxyhexanoyl-CoA from an unspecified source and of an unspecified structure, or the FaoAB complex from *Pseudomonas putida* of unspecified identity.

Appellant describes by incorporation through reference genes encoding the FaoAB multimeric complex from *Pseudomonas fragi* and the FadAB (i.e. FaoAB) multimeric complex

from *E. coli* that possess an activity that epimerizes S-3-hydroxyhexanoyl-CoA to R-3-hydroxyhexanoyl-CoA.

Appellant does not describe any genes encoding fatty acid biosynthetic enzymes from *Norcardia salmonicolor* or the enzymes; or any gene encoding an enzyme that epimerizes S-3-hydroxyhexanoyl-CoA to R-3-hydroxyhexanoyl-CoA or the enzyme; or genes encoding the FaoAB from *Pseudomonas putida* or the enzyme complex.

The Federal Circuit has recently clarified the application of the written description requirement to inventions in the field of biotechnology. The court stated that, "A description of a genus of cDNAs may be achieved by means of a recitation of a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to members of the genus, which features constitute a substantial portion of the genus." *See University of California v. Eli Lilly and Co.*, 119 F.3d 1559; 43 USPQ2d 1398, 1406 (Fed. Cir. 1997).

Appellants fail to describe a representative number of fatty acid biosynthetic enzymes from *Norcardia salmonicolor* or genes thereof; or genes encoding an enzyme that epimerizes S-3-hydroxyhexanoyl-CoA to R-3-hydroxyhexanoyl-CoA or enzymes thereof; or genes encoding the FaoAB from *Pseudomonas putida* or the enzyme complex. Appellants only describe, by incorporation through reference, genes encoding the FaoAB multimeric complex from *Pseudomonas fragi* and the FadAB multimeric complex from *E. coli* that possess an activity that epimerizes S-3-hydroxyhexanoyl-CoA to R-3-hydroxyhexanoyl-CoA known in the art.

Further, Appellants fail to describe structural features common to members of the claimed genera of fatty acid biosynthetic enzymes from *Norcardia salmonicolor*; enzymes that

epimerize S-3-hydroxyhexanoyl-CoA to R-3-hydroxyhexanoyl-CoA or genes encoding the FaoAB from *Pseudomonas putida* that would distinguish them.

Hence, Appellants fail to meet either prong of the two-prong test set forth by *Eli Lilly*. Furthermore, given the lack of description, in the art or in Appellants' specification of the necessary elements essential for the activity of the claimed genera of enzymes, it remains unclear what features identify them. Since the genus of fatty acid biosynthetic enzymes from *Norcardia salmonicolor* or the genes thereof; or genes encoding an enzyme that epimerizes S-3-hydroxyhexanoyl-CoA to R-3-hydroxyhexanoyl-CoA or enzymes thereof; or genes encoding the FaoAB from *Pseudomonas putida* or the enzyme complex itself has not been described by specific structural features, the specification fails to provide an adequate written description to support the breath of the claims.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the Appellant regards as his invention.

Claim 39 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Appellant regards as the invention.

Claim 39 recites the limitation "wherein the enzymes" in line 1. There is insufficient antecedent basis for this limitation in the claim. Claim 38 is drawn to a single enzyme, it is not clear which enzyme is being claimed in claim 39.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 7, 10, 14, 16, 18-21, 36 and 38 are rejected under 35 U.S.C. 102(b) as being anticipated by Fukui *et al.* (J. of Bacteriol. Vol. 179: pp. 4821-4830, 1997).

The claims are drawn to a method for the biological production of polyhydroxyalkanoate containing 3-hydroxyhexanoate, comprising providing genetically engineered bacteria that express a 3-ketothiolase gene that converts butyryl-CoA and acetyl-CoA to 3-ketohexanoyl-CoA; a reductase gene that encodes an acetoacetyl-CoA reductase enzyme that converts 3-ketohexanoyl-CoA to 3-hydroxyhexanoyl-CoA; and a PHA polymerase gene encoding an enzyme that polymerizes 3-hydroxybutyryl CoA and 3-hydroxyhexanoyl CoA; wherein the bacteria can utilize butyrate or butanol and the bacteria will produce polyhydroxybutyrate-co-3-hydroxyhexanoate (claim 1); wherein the polymerase is from a bacterium selected from the group consisting of *Aeromonas caviae, C. testosterone, T. pfenigii, C. vinosum, B. cereus, N. Carolina, N. salmonicolor, R. rubber R. rhodocrous,* and *R. rubrum* (claim 7); or the bacteria comprise a gene encoding a 3-hydroxyacyl-ACP-coenzyme-A transferase (claim 10); or the bacteria are selected from the group consisting of *E. coli, Klebsiella, Ralstonia, Alcaligenes, Pseudomonas,* and *Azotobacter* (claim 14); or the bacteria further expresses a gene encoding a D-specific enoyl-CoA hydratase (claim 16); or the bacteria expresses one or more fatty acid biosynthetic enzymes; wherein the fatty acid biosynthetic enzymes are selected from the group

consisting of 3-hydroxyacyl-ACP-coenzyme-A transferase, acyl-ACP thioesterase, and acyl-CoA synthase (claims 18-21) or form a complex (claim 36) or epimerize S-3-hydroxyhexanoyl-CoA to R-3-hydroxyhexanoyl-CoA (claim 38).

The following references are provided as evidence in the discussion below that *R. eutropha* (*A. eutrophus*) express the enzymes of the claims:

Schubert P. *et al.*, (J. of Bacteriology 1988; Vol. 170, No. 12, p. 5837-5847);

U.S. Patent 6,586,658 (Peoples *et al.*);

Doi Y. *et al.* Applied Microbiology and Biotechnology; 1988, Vol. 28 pp. 330-334;

Kremer, L. *et al.* J. of Biol. Chem. July 27, 2001; Vol. 276, No. 30, pp.27967-27974;

GenBank Accession gi: 113527306 for a FabD gene encoding the *Ralstonia eutropha*;

GenBank Accession gi: 73539398 for an acyl CoA synthase (i.e. synthetase) gene from *Ralstonia eutropha*;

Concise Encyclopedia Biochemistry 2nd. Ed. Scott, T. and Eagleson, M.; Walter de Gruyter: Berlin-New York 1988 for structure of malonyl-CoA p.197;

and from Appellants's specification:

page 11 lines 13-16 and 22-24 for Haywood G.W. *et al.* FEMS Microbiology Letters; 1988, Vol. 52 pp. 91-96; and Haywood G.W. *et al.* FEMS Microbiology Letters; 1988, Vol. 52 pp. 259-264;

example 4 pages 24-25 recites Fukui *et al.*, J. of Bacteriol. Vol. 179: 4821-4830, 1997 for the source of the D-specific hydratase of claim 16.

page 14 line 26 to page 15 line 1 that points to *R. eutropha* (*A. eutrophus*) as a source of an (FaoAB) complex of claim 36 that has the epimerase activity of claim 38;

Fukui discloses a method for producing polyhydroxyalkanoates containing 3-hydroxhexanoate by transformation of a bacteria *Alcaligenes eutrophus* (now called *Ralstonia eutropha* of claim 14) with a gene (*phaC*) from *Aeromonas caviae* (claim 7) encoding a PHA (polyhydroxyalkanaote) synthase (i.e. polymerase) that polymerizes polyhydroxybutyrate-co-3-hydroxyhexanoate i.e. HB-co-3HH (claim 1); (See Fukui abstract especially the last 2 lines; page 4828 in Table 3 *A. eutrophus* transformed with pJRDEE32d13 with respect to HHx copolymer production; and page 4829 column 2, lines 37-44); and production of polyhydroxyalkanoates containing 3-hydroxhexanoate by transformation of *A. eutrophus* and *Pseudomonas putida* transformed with plasmids pJRDEE32 and pJRDEE50 both plasmids of which comprise the *PhaC* polymerase that polymerizes polyhydroxybutyrate-co-3-hydroxyhexanoate (HB-co-3HH) and ORF3 (see Fukui *et al.* on page 4829 in column 1 Table 5) encoding an R-specific enoyl-CoA hydratase or a D-specific enoyl-CoA hydratase as defined in Example 4 of Appellants' specification on pages 24-25 that recites Fukui *et al.*, J. of Bacteriol., 1997, Vol. 179: pp. 4821-4830, as the source of the *phaJ* gene encoding the D-specific hydratase (claim 16);

wherein *R. eutropha* expresses the following enzymes:

1) a 3-ketothiolase gene (claim 1) that condenses butyryl-CoA and acetyl-CoA to 3-ketohexanoyl-CoA (see Haywood G.W. *et al.* FEMS Microbiology Letters; 1988, Vol. 52 pp. 91-96 on page 93 section 4.3 to page 94 end of section and in Table 2 that shows "thiolase B" specificity for 3-ketohexanoyl-CoA; U.S. Patent 6,586,658 column 21 lines 10-21 that points to

using this gene encoding this enzyme because it condenses butyryl-CoA and acetyl-CoA to 3-ketohexanoyl-CoA; and Appellants' specification page 11 lines 19-24);

2) a reductase gene (claim 1) that encodes an acetoacetyl-CoA reductase enzyme that converts 3-ketohexanoyl-CoA to 3-hydroxyhexanoyl-CoA (see Haywood G.W. *et al.* FEMS Microbiology Letters; 1988, Vol. 52 pp. 259-264; on page 262, column 2 in Table 1; and Appellants' specification on page 11 lines 22-24),

3) an acyl CoA synthase (claims 20-21), See Schubert *et al.* page 5845 in figure 2 see enzyme number 1 for the acyl Co-A synthetase or synthase; and GenBank Accession gi: 73539398 for a gene encoding the *Ralstonia eutropha* acyl CoA synthase also referred to as a synthetase;

4) fatty acid biosynthetic enzymes (claims 10, 18-20) see Kremer, L. *et al.* J. of Biol. Chem. July 27, 2001; Vol. 276, No. 30, pp.27967-27974 lines 1-7 of the Abstract; and GenBank Accession gi: 113527306 for a FabD gene encoding the *Ralstonia eutropha* malonyl-ACP-coenzyme-A transferase; and the structure of Malonyl-CoA showing that carbon 3 is hydroxylated meeting the limitation of a 3-hydroxyacyl compound found in claims 10, 19 and 20 (Concise Encyclopedia Biochemistry 2nd. Ed. Scott, T. and Eagleson, M.; Walter de Gruyter: Berlin-New York 1988 for structure of malonyl-CoA p.197).

5) a D-specific enoyl-CoA hydratase (claim 16) see Schubert in Figure 2 enzyme number 6 for the D-specific enoyl-CoA hydratase; and from Appellants' specification example 4 pages 24-25 that recites Fukui *et al.*, J. of Bacteriol. Vol. 179: 4821-4830, 1997 supra for the source of the D-specific hydratase of claim 16.

6) for the utilization of butyrate (claim 1) see Schubert *et al.* page 5845 in figure 2 on the left side just before enzyme 1 for utilization of butyrate, i.e. butyrate being fed into the pathway of PHB synthesis; and Doi Y. *et al.* Applied Microbiology and Biotechnology; 1988, Vol. 28 pp. 330-334 for utilization of butyrate; in summary lines 1-5;

7) *R. eutropha* (*A. eutrophus*) as a source of an (FaoAB) complex (claim 36) that has the claimed epimerase activity (claim 38); Appellants' specification on page 14 line 26 to page 15 line 1;

please note that Claim 21 is interpreted as either an acyl ACP thioesterase "or" an acyl CoA synthetase or both an acyl ACP thioesterase and an acyl CoA synthetase because of the limitation of Claim 18 from which it depends recites "one or more fatty acid biosynthetic enzymes"; and thus the alternative "or" is an acceptable interpretation, and thus the reference teaches all the limitations of claims 1, 7, 10 14, 16, 18-21, 36 and 38.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 6-7, 10, 14, 16-21 and 35-39 are rejected under 35 U.S.C. 103(a) as being unpatentable over Fukui *et al.* (J. of Bacteriol. 1997; Vol. 179: p. 4821-4830), in view of Macharenas *et al.* (U.S. Patent 5,470,727 issued 28 November 1995); in further view of Schubert P. *et al.*, (J. of Bacteriology 1988; Vol. 170, No. 12, p. 5837-5847); Boynton Z. *et al.* (J. of

Bacteriology June 1996, Vol. 178, No. 11, p. 3015-3024); U.S. Patent 5,512,482 issued to Voelker *et al.* April 30, 1996 and Appellants' specification.

Claims are drawn to a method for the biological production of polyhydroxyalkanoate containing 3-hydroxyhexanoate, comprising providing genetically engineered bacteria that express a 3-ketothiolase gene that converts butyryl-CoA and acetyl-CoA to 3-ketohexanoyl-CoA; a reductase gene that encodes an acetoacetyl-CoA reductase enzyme that converts 3-ketohexanoyl-CoA to 3-hydroxyhexanoyl-CoA; and a PHA polymerase gene encoding an enzyme that polymerizes 3-hydroxybutyryl CoA and 3-hydroxyhexanoyl CoA; wherein the bacteria can utilize butyrate or butanol and the bacteria will produce polyhydroxybutyrate-co-3-hydroxyhexanoate (claim 1); wherein the PHA polymerase transgene is incorporated into the bacterial chromosomal DNA (claim 6); wherein the polymerase is from a bacterium selected from the group consisting of *Aeromonas caviae, C. testosterone, T. pfenigii, C. vinosum, B. cereus, N. Carolina, N. salmonicolor, R. rubber R. rhodocrous,* and *R. rubrum* (claim 7); or the bacteria are selected from the group consisting of *E. coli, Klebsiella, Ralstonia, Alcaligenes, Pseudomonas,* and *Azotobacter* (claim 14); or the bacteria further expresses a gene encoding a D-specific enoyl-CoA hydratase (claim 16); or express three enzymes from *C. acetobutylicum* that form butyryl-CoA (claim 17); or the bacteria expresses one or more fatty acid biosynthetic enzymes; wherein the fatty acid biosynthetic enzymes are selected from the group consisting of 3-hydroxyacyl-ACP-coenzyme-A transferase, acyl-ACP thioesterase, and acyl-CoA synthase (claims 10, 18-21); or express fatty acid biosynthetic enzymes from *E. coli* or *Norcardia salmonicolor* (claims 35 and 37); or form a complex (claim 36); or epimerize S-3-

hydroxyhexanoyl-CoA to R-3-hydroxyhexanoyl-CoA (claim 38); or comprise the FaoAB complex of *P. putida* (claim 39).

Fukui is discussed supra.

Fukui discloses a method for producing polyhydroxyalkanoates containing 3-hydroxhexanoate by transformation of a bacteria *Alcaligenes eutrophus* (now called *R. eutropha*) with a gene (*phaC*) from *Aeromonas caviae* encoding a PHA synthase (i.e. polyhydroxybutyrate-co-3-hydroxyhexanoate polymerase) that resulted in levels of production of polyhydroxybutyrate-co-3-hydroxyhexanoate (i.e. HB-co-3HHx polymer) that accumulated to 96% of total cellular dry weight when grown on hexanoate (C6) or octanoate (C8) (i.e. hexanoic and octanoic fatty acids) and the mol fraction of HB and HHx in the co-polyester reached 80% HB and 20% HHx, and when grown on hexanoate (i.e. C6 hexanoic fatty acid), HB-co-3HHx polymer accumulated to 72% of total cellular dry weight and the mol fraction of HB and HHx in the co-polyester reached 50% (See Fukui abstract, especially the last 2 lines; page 4828 Table 3 in plasmid row pJRDEE32d13 (*A. eutrophus* transformed with pJRDEE32d13), and page 4829 column 2, lines 37-42); and production of HB-co-3HHx by transformation of *A. eutrophus* and *Pseudomonas putida* transformed with pJRDEE32 and pJRDEE50 both of which comprise the *PhaC* polymerase that polymerizes polyhydroxybutyrate-co-3-hydroxyhexanoate (HB-co-3HH) and ORF3 (see Fukui in Table 3 and page 4829 column 2, lines 37-44 as well as page 4829 in Table 5 and Figure 4) encoding an R-specific enoyl-CoA hydratase or a D-specific enoyl-CoA hydratase as defined in Example 4 of Appellants' specification on pages 24-25 that recites Fukui *et al.*, J. of Bacteriol., 1997, Vol. 179: pp. 4821-4830 supra as the source of the *phaJ* gene encoding the D-specific hydratase of claim 16;

Fukui does not teach incorporation of the *PHA* polymerase (synthase) gene into the bacterial chromosome; expression of three enzymes from *C. acetobutylicum* that form butyryl-CoA; expression of fatty acid biosynthetic enzymes from *E. coli* or *Norcardia salmonicolor*; expression of an acyl ACP-thioesterase; or expression of the FaoAB complex of *P. putida.*

Mascarenhas teaches that chromosomal integration of genes encoding heterologous peptides would be advantageous as an alternative means for expression of foreign proteins in bacterial host cells because plasmids or multi copy vectors are unstable and require some means of selection such as antibiotics in order to maintain their expression (see column 1, lines 10-32) and also teaches a method of chromosomal integration of a foreign gene (see the Example in columns 7 and 8; and Claim 1).

Boynton teaches three enzymes from *C. acetobutylicum* that form butyryl CoA (claim 17) transformed into *E. coli* and *A. acetobutylicum* in the abstract.

Schubert teaches that *Alcaligenes eutrophus* (now called *R. eutropha*) express an acyl CoA synthase (i.e. synthetase), fatty acid biosynthetic enzymes and a D-specific enoyl-CoA hydratase; and utilizes butyrate on page 5845 in figure 2 for the acyl Co-A synthetase see enzyme number 1; for the fatty acid beta oxidation enzymes see enzyme number 8; for the D-specific enoyl-CoA hydratase see enzyme number 6 as well as page 5845 column 1 lines 1-5; and for the utilization of butyrate see in Figure 2 on the left side just before enzyme 1 for utilization of butyrate, i.e. butyrate being fed into the pathway of PHB synthesis.

U.S. Patent 5,512,482 to Voelker discloses an acyl ACP thioesterase (claims 18-21) and their use in production of polyhydroxybutyrate-based plastics in bacteria (column 1 lines 25-49; column 24 Table 6 activities on C6-C14 ACP fatty acid conjugates).

Appellants's specification discloses inherent features of *R. Eutropha*;

R. Eutropha comprises a 3-ketothiolase gene (claim 1) that converts butyryl-CoA and acetyl-CoA to 3-ketohexanoyl-CoA and

a reductase gene that encodes an acetoacetyl-CoA reductase enzyme (claim 1) that converts 3-ketohexanoyl-CoA to 3-hydroxyhexanoyl-CoA and were known in the art; see Appellants' specification on page 11 lines 13-16 and 22-24 for Haywood G.W. *et al.* FEMS Microbiology Letters; 1988, Vol. 52 pp. 91-96 and Haywood G.W. *et al.* FEMS Microbiology Letters; 1988, Vol. 52 pp. 259-264; and

the source of the D-specific hydratase of claim 16 in Appellants' specification in example 4 on pages 24-25 that Fukui *et al.*, J. of Bacteriol. Vol. 179: 4821-4830, 1997;

R. eutropha (*A. eutrophus*) as a source of an (FaoAB) complex of claim 36 that has the epimerase activity of claim 38; on page 14 in Appellants' specification line 26 to page 15 line 1;

on page 15 lines 18-23 and on page 25 lines 10-16 especially lines 13-16 of Appellants' specification that a gene encoding a 3-hydroxyacyl-ACP-coenzyme A transferase (claim 10) is inherent to all bacterial organisms that produce PHA from oxidized carbon sources such as *R. eutropha* and can easily be isolated as an alternative to the activities of the 'widely known' (page 15 line 20) acyl ACP thioesterase and acyl CoA synthase for directing products of fatty acid metabolism from the hydroxyacyl-ACP to the acyl-CoA derivative for the production of polyhydroxybutyrate-co-3-hydroxyhexanoate.

It would have been obvious to modify the invention of Fukui to substitute the expression of a foreign gene on a plasmid for the expression of the foreign gene incorporated into bacterial chromosome as taught by Mascarenhas; and to further include gene encoding the acyl ACP-

thioesterase of Voelker (the '482 Patent) that terminates fatty acid elongation to produce C6-C10 fatty acids for incorporation into HB-co-3HHx polymer demonstrated by Fukui when growing *R. eutropha* transformed with the PHA polymerase from *A,caiave* on hexanoate (C6) or octonoate (C8) fatty acids; or to substitute or augment the three enzymes that form butyryl-CoA inherent to *R. eutropha* as taught by Schubert with the three enzymes from *C. acetobutylicum* that form butyryl CoA as taught by Boynton; or to substitute or augment the enzymes of the fatty acid biosynthesis pathway inherent to *R. eutropha*, as discussed supra in the rejection under 102(b), with the genes encoding homologous enzymes from *E. coli*, *Norcardia salmonicolor*, or *Pseudomonas putida* (35, 37 and 39). One of ordinary skill in the art would have recognized what is generally known in the art, that plasmid based bio-reactor systems require the extra burden of maintaining the plasmid that contains the transgene under a regime of selection and that bio-reactor systems suffer from unstable expression because of plasmid loss when selection fails and that this problem could be overcome by using chromosomally integrated genes and one of ordinary skill would have been motivated further by the teachings of Mascarenhas that chromosomal integration of foreign genes into the bacterial chromosome would allow for stable expression of a recombinant protein without maintaining selection; and furthermore recognized and found motivation by the fact that including an acyl ACP-thioesterase or any combination of enzymes that convert 3-hydroxyacyl-ACP to 3-hydroxyacyl-CoA (claims 10 and 18-21) would provide substrate for the PHA polymerase taught by the methodology of Fukui without the requirement of adding hexanoate or octonoate, thereby improving the efficiency of the process of Fukui; and by the knowledge common in the art that *Alcaligenes eutrophus* (i.e. *Ralstonia eutropha*) comprises multiple endogenous PHA biosynthetic genes that express the 3-

ketothiolase that converts butyryl-CoA and acetyl-CoA to 3-ketohexanoyl-CoA, the acetoacetyl-CoA reductase enzyme that converts 3-ketohexanoyl-CoA to 3-hydroxyhexanoyl-CoA, a D-specific enoyl-CoA hydratase, three fatty acid enzymes that form butyryl-CoA as well as one or more fatty acid biosynthetic enzymes and an acyl CoA synthase; genes of which are already integrated into the chromosome of *A. eutrophus* as taught by Schubert; and further motivated by the success of Fukui in producing polyhydroxybutyrate-co-3-polyhydroxyhexanoate from *A. eutrophus* and *Pseudomonas putida* transformed only with the *PHA* synthase gene from *A. eutrophus* (now called *R. eutropha*) demonstrating that *R. eutropha* cells already have the ability to supply 3-hydroxyhexanoate to the heterologous PHA polymerase (see Fukui page 4829 column 2, lines 37-44); and the success of Mascarenhas in stably expressing chromosomally integrated foreign genes; and that one of ordinary skill would have a reasonable expectation of success given the success of the methodology taught by Fukui for the biological production of polyhydroxyalkanoate containing 3-hydroxyhexanoate and of the success of Mascarenhas in stable chromosomal expression; wherein one of ordinary skill in the art would have recognized that *A. eutrophus* (now called *R. eutropha*) is able to both provide 3-hydroxybutyryl-CoA and also convert fatty acids to 3-hydroxyhexanoyl-CoA for biosynthesis of polyhydroxybutyrate-co-3-hydroxyhexanoate; and to further incorporate into *R. eutropha*, genes that express enzymes fatty acid biosynthetic enzymes from *E. coli* or *Norcardia salmonicolor*, or convert fatty acids to butyryl-CoA, or that form a complex or epimerize S-3-hydroxyhexanoyl-CoA to R-3-hydroxyhexanoyl-CoA, or genes that encode enzymes that comprise the FaoAB complex of *P. putida*; or a gene or genes encoding an activity that converts acyl ACP into acyl CoA, a reaction catalyzed by an ACP/CoA transferase all of which were either known in the art

or easily available to one of ordinary skill in the art; to include those features is obvious given the lack of criticality, given that the levels of HB-co-3HHx polymer in transformed *R. eutropha* as taught by Fukui accumulated to 96% of total cellular dry weight when grown on octanoate and the mol fraction of HB and HHx in the co-polyester reached 80% HB and 20% HHx; and when grown on hexanoate polymer accumulated to 72% of total cellular dry weight and the mol fraction of HB and HHx in the co-polyester reached 50% (See Fukui abstract, especially the last 2 lines; page 4828 Table 3 plasmid row pJRDEE32d13, and page 4829 column 2, lines 37-42). Moreover one of ordinary skill would have recognized that Fukui *et al.* showed that the biosynthetic pathways of polyhydroxybutyrate and polyhydroxy hexanoate could be brought together to produce polyhydroxybutyrate-co-3-hydroxyhexanoate (i.e. HB-co-3HHx) since *R. eutropha* was able to present the substrate 3-hydroxyhexanoate to the heterologous PHA polymerase from *A, caviae* (page 4829 column 2, lines 37-42).

(10) Response to Argument

Response to Rejection under 102

Appellant asserts that Fukui *et al.* used different pathways and different substrates than those set forth in the Examples (response page 10 1st full paragraph under Fukui). The features of the Examples that Appellant relies upon in their arguments such as transformed *E. coli* are not reflected in their claims. Although the claims are interpreted in light of the specification, limitations from the specification are not read into the claims. See *In re Van Geuns*, 988 F.2d 1181, 26 USPQ2d 1057 (Fed. Cir. 1993). Claim 1 recites "wherein the bacteria can utilize butyrate or butanol". This limitation has no patentable weight because it points to an inherent property of the bacteria and not a feature of the method such as an active method step. If the

claim we rewritten to recite "wherein the bacteria are fed butanol or butyrate" the Examiner would consider withdraw of the rejections.

Claim 1 does not set forth any transformation but merely recites that the bacteria is genetically engineered to produce the PHBH polymer and relies upon the dependent claims that recite enzymes from specific species to insinuate transformation. In general, the claims only recite that the bacteria express the enzymes. There is nothing in the claims stating that the bacteria are actually transformed as they are in the Examples, and since genetically engineered also encompasses engineering through mutagenesis, one can only surmise through inference that they must be transformed because there are enzymes from different species recited in the dependent claims. Appellant asserts that there is no basis in fact in Schubert for the thiolase elongation reaction recited in claim 1 (response page 12). Appellants' attention is directed to Appellants's specification: for the thiolase and reductase activities recited in claim 1 evidence is provided by the following references incorporated into Appellants' specification on page 11 lines 13-16 and page 11 lines 22-24 for Haywood G.W. *et al.* FEMS Microbiology Letters; 1988, Vol. 52 pp. 91-96; and Haywood G.W. *et al.* FEMS Microbiology Letters; 1988, Vol. 52 pp. 259-264.

Appellant asserts on page 11 lines 3-4 that the method provides substrates sequentially utilized by each enzyme in the pathway. Appellants' assertions are not found persuasive because those features are not recited in the Examples of the specification. None of Appellants' examples (i.e Examples 1-6 pages 19-26) in the specification teach or practice utilization of a 3-ketothiolase that condenses butyryl-CoA and acetyl-CoA in a pathway to produce PHA polymer. Appellants's examples are drawn to a 3-ketothiolase from *R. eutropha* encoded by the phaA gene on page 21 in Example 2 in lines 26-28 see plasmid pSU18-AB1, which is the Thiolase A gene

discussed in Haywood G.W. *et al.* FEMS Microbiology Letters; 1988, Vol. 52 pp. 91-96, the product of which does not condense butyryl-CoA and acetyl-CoA. Further in Example 5, plasmid pSU18-AB1 is used in the only example that shows production of PHBH copolymer when grown on butyrate. Clearly, Appellants' methodology does not practice sequential utilization of the enzymes because the first step of the sequence recited in Claim 1 is missing in Examples 1-6 of the specification. The missing 3-ketothiolase activity is an inherent feature of *E. coli* (see Slater S. *et al.* J. of Bacteriology; April 1998, Vol. 180, No. 8; pp. 1979-1987 beginning on page 1979 in column 1 last paragraph to end of introduction).

Appellant asserts on page 11 lines 3-4 that the method provides that the bacteria are grown under conditions wherein the polyhydroxybutyrate-co-3-hydroxyhexanoate is produced. Appellants' assertions are not found persuasive because those features are not recited in the claims. The claims recite no growth conditions whatsoever, most notably as what to feed the bacteria.

Further in response to Appellants' assertions on page 11 lines 5-6 that Fukui does not provide the enzymes for utilization of butanol or butyrate see arguments supra and under 102(b).

In response to Appellants' assertions on page 11 lines 7-10 Appellants' attention is directed to Appellants' specification for further evidence for inherently expressed enzymes in *R. eutropha*; in example 4 pages 24-25 recites Fukui *et al.*, J. of Bacteriol. Vol. 179: 4821-4830, 1997 for the source of the D-specific hydratase of claim 16; on page 15 lines 18-23 and on page 25 lines 10-16 especially lines 13-16 of Appellants' specification that a gene encoding a 3-hydroxyacyl-ACP-coenzyme A transferase (claim 10) is inherent to all bacterial organisms that produce PHA from oxidized carbon sources (such as *R. eutropha*) and can easily be isolated as

an alternative to the activities of the widely known acyl ACP thioesterase and acyl CoA synthase; and on page 14 line 26 to page 15 line 1 that points to *R. eutropha* (*A. eutrophus*) as a source of an (FaoAB) complex of claim 36 that has the epimerase activity of claim 38.

Appellant asserts that Fukui does not disclose the use of fatty acid enzymes for the production of PHBH and thus provides further evidence that claims 10, 18-21 and 35-39 are novel (response page 13). Appellant does not claim "the use of the enzymes" in the claims but rather recites that the bacteria express the enzymes of the claims. This broadly stated claim language reads upon endogenous bacterial enzyme expression as well as heterologous expression or transgene expression and as such the material provided as evidence in the rejection meets the legal standard for evidence as presented on page 9 of Appellants' response.

Appellant asserts (on page 12 last paragraph to page 13 line 3) that the Schubert reference does not disclose a thiolase that condenses butyryl-CoA and acetyl-CoA is inherent to *R. eutropha* (*A. eutrophus*). Appellants' remarks contradict the teachings in Appellants' specification and the information presented in Appellants' IDS that there are two 3-ketothiolases in *R. eutropha* and that the second 3-ketothiolase does condense butyryl-CoA and acetyl-CoA. In fact the second ketothiolase enzyme (i.e. Thiolase B or enzyme B) is described by Haywood G.W. *et al.* FEMS Microbiology Letters; 1988, Vol. 52 pp. 91-96 on page 94 in column 1 as having a broad substrate range and a lower K_m than "Enzyme A" or "Thiolase A" indicating that Thiolase B would be active at lower concentrations of substrate, recognized by one of ordinary skill in the art as levels of substrate that reflect 'physiological concentrations' found within the cell. Moreover, in none of Appellants' examples (i.e Examples 1-6 pages 19-26) in the specification do they teach or practice utilization of a 3-ketothiolase that condenses butyryl-CoA

and acetyl-CoA in a pathway to produce PHA polymer. Appellants's examples are drawn to a 3-ketothiolase from *R. eutropha* encoded by the phaA gene on page 21 in Example 2 in lines 26-28 and Example 5 see plasmid pSU18-AB1, which encodes the Thiolase A discussed supra, the product of which does not condense butyryl-CoA and acetyl-CoA.

Response to Rejection under 103

Appellant asserts that a *prima facie* case of obviousness is not possible when the claimed invention achieves more than any or all of the prior art references (response page 14 bottom of page). This argument however is not persuasive because clearly Fukui more than exceeds the levels of production of PHBH recited by Appellant in their examples (see rejection under 103 supra).

Appellant asserts on page 16 that Fukui does not disclose bacteria encoding genes that enable the production of PHBH from butyryl-CoA and acetyl-CoA is misplaced because Appellant does not recite that limitation in the claims. Rather, Appellant only recites that the enzymes are expressed and that they have a certain activity or that the gene is incorporated into the chromosome that reads upon any endogenous PHA polymerase. Again Appellant states that Mascarenhas does not teach that the integrated gene works in a pathway, however 'a pathway' is not a limitation in the claims.

Appellant asserts that they are the first to show genes from two separate pathways (transformed) in an organism that showed polymer production (response page 21 3rd paragraph). This is not found persuasive because Fukui clearly teaches that the transformation of *R. eutropha* with the PHA polymerase gene and the hydratase gene (i.e. ORF 3) from *A. caviae* brings together three pathways; the PHBH (polyhydroxyutyrate-co-3-hydroxyhexanoate) pathway from

A.caviae represented by the PHA polymerase, the PHB (polyhydroxybutyrate) pathway inherent to *R. eutropha*, and the fatty acid oxidation pathway from *A. caviae* represented by the hydratase of ORF3 that supplies both short (C4) and medium (C6) chain length copolymers i.e. 3-hydroxybutyrate and 3-hydroxyhexanoate (see Fukui page 4829 lines 49-56).

In conclusion, with respect to Appellants' remarks concerning the thiolase on page 21, Appellants' attention is directed to arguments supra and the new grounds of rejection.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

For the above reasons, it is believed that the rejections should be sustained.

This examiner's answer contains a new ground of rejection set forth in section **(9)** above. Accordingly, appellant must within **TWO MONTHS** from the date of this answer exercise one of the following two options to avoid *sua sponte* **dismissal of the appeal** as to the claims subject to the new ground of rejection:

(1) **Reopen prosecution.** Request that prosecution be reopened before the primary examiner by filing a reply under 37 CFR 1.111 with or without amendment, affidavit or other evidence. Any amendment, affidavit or other evidence must be relevant to the new grounds of rejection. A request that complies with 37 CFR 41.39(b)(1) will be entered and considered. Any request that prosecution be reopened will be treated as a request to withdraw the appeal.

(2) **Maintain appeal.** Request that the appeal be maintained by filing a reply brief as set forth in 37 CFR 41.41. Such a reply brief must address each new ground of rejection as set forth in 37 CFR 41.37(c)(1)(vii) and should be in compliance with the other requirements of 37 CFR

41.37(c). If a reply brief filed pursuant to 37 CFR 41.39(b)(2) is accompanied by any amendment, affidavit or other evidence, it shall be treated as a request that prosecution be reopened before the primary examiner under 37 CFR 41.39(b)(1).

Extensions of time under 37 CFR 1.136(a) are not applicable to the TWO MONTH time period set forth above. See 37 CFR 1.136(b) for extensions of time to reply for patent applications and 37 CFR 1.550(c) for extensions of time to reply for ex parte reexamination proceedings.

Respectfully submitted,
Russell P. Kallis Ph.D.
March 16, 2007

A Technology Center Director or designee must personally approve the new ground(s) of rejection set forth in section (9) above by signing below:

Christopher Low

Director TC1600 (acting)
for GEORGE C. ELLIOTT, DIRECTOR
TECHNOLOGY CENTER 1600

Conferees:

Anne-Marie Grunberg

ANNE MARIE GRUNBERG
SUPERVISORY PATENT EXAMINER

Joseph Woitach

JOSEPH WOITACH, PH.D.
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Russell Kallis whose telephone number is (571) 272-0798. The examiner can normally be reached on M-F 8:30-5.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anne Marie Grunberg can be reached on (571) 272-0975. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Russell Kallis Ph.D.
March 16, 2007

RUSSELL P. KALLIS, PH.D.
PRIMARY EXAMINER